bio-techne®

Application Note

Genomic Profiling, Advantages of Liquid Biopsy-Based Biomarkers

Studies from multiple partnerships with Exosome Diagnostics, a Bio-Techne brand, highlights capabilities in patient stratification, exosome isolation and RNA profiling. Specifically the focus of the following studies will be around stratifying glioblastoma patients to predict responses to potential therapies.

Exosome Diagnostics can:

1. Accurately characterize and stratify patient populations.
2. Get versatility in target analytes to better characterize populations.
3. Analyze biofluids that can provide minimally and non-invasive options to obtain critical information.
4. Create customized experimental approaches based on over a decade of exosome experience.

Contents

Overview

Capabilities:

Drug target engagement, risk assessment, diagnostics, patient stratification, etc.

Sample:

Data demonstrates feasibility in serum/plasma, CSF, urine and other biofluids.

Analyte:

RNA, protein, nucleic acids, and more allowing studies in metabolomics, lipidomics and glycomics.

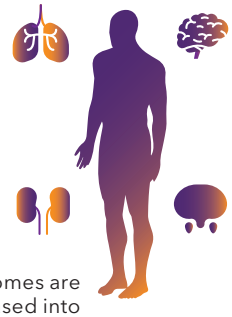
Expertise:

Exosome Diagnostics has extensive intellectual property related to exosome-based diagnostics, is the only company with an exosome based diagnostic and the longest running company providing solutions around exosome diagnostics.

Quality:

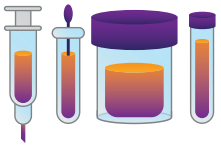
Bio-Techne is able to provide clinical workflow solutions with CGMP and GCLP capabilities.

Exosome Isolation & Genomic Profiling



Exosomes are released into human biofluids

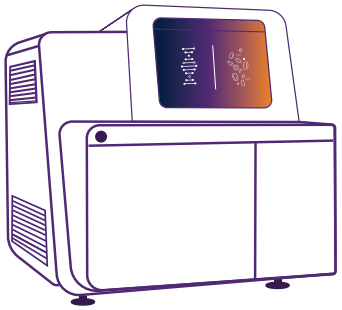
Exosome Release



Exosomes are collected from various biofluids: Saliva, Plasma/Serum, CSF and Urine.

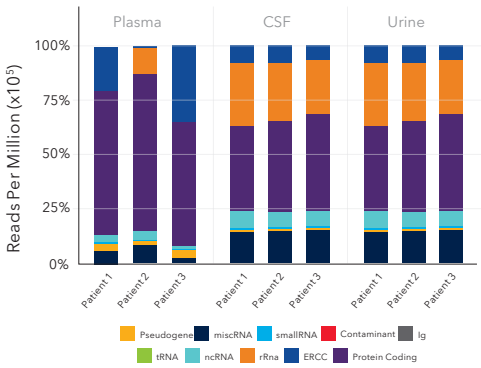
Exosome Isolation

Exosomes are stable vesicles of enriched information that can be isolated from biofluids via patented approaches.



Exosomes are analyzed by various downstream methods including RNAseq whole transcriptome sequencing.

Data Interpretation



Exosome RNA analysis enables real-time longitudinal monitoring of cellular process.

Clinical Interpretation

Clinical correlation, biomarker discovery, patient stratification and characterization, longitudinal monitoring.

The Power of Exosome Analysis

FIGURE 1 Summary diagram. Exosomes are actively released from living tissues and can be captured from biofluids such as blood, urine, cerebro spinal fluid (CSF) and saliva. The exosome compartment stabilizes the RNA transcriptome and if isolated correctly, the entire transcriptome can be mapped (RNAseq). From as little as 0.5 ml of plasma we can see over 30,000 of the 56,000 RNA targets in gencode. This can enable mapping of RNA pathways dysregulated by the disease or by a particular drug treatment and enables stratification of responders, non-responders etc.

Introduction

Tremendous diagnostic power resides in exosomes as demonstrated by numerous peer-reviewed studies in various areas including cancer, cardiovascular disease, transplant monitoring and specifically in neurology.[1-5] Exosomes are used as a clinical diagnostics, most notably the ExoDx Prostate test that has helped over 70,000 prostate cancer patients to date.[6-7] Exosomes are powerful diagnostic tools:

- Number in the billions per milliliter of plasma, urine and other biofluids.
- Originate from almost every cell in the body regardless of disease state.
- Cross the blood-brain barrier.
- Are rich sources of RNA, proteins, and other nucleic acids.
- Are actively released by living cells.
- Allow characterization with multi-analyte analyses and native and modified states (e.g.methylation).
- Able to be separated by tissue-specific markers to analyze an enriched signature.

Background and Results

Nearly 1 in 6 people globally are suffering from neurological disorders and brain related cancers including glioblastoma (GBM), Alzheimer's, Parkinson disease, stroke, multiple sclerosis, epilepsy, brain injuries and neuroinfections.[8] Five-year survival rates for patients with neurological-related cancer remains relatively stagnant compared to other cancers, changing very little from 1975-1977 to 2006-2012.[9] Furthermore, as the population increases and the average life-spans increases the prevalence of cancer and neurological disorders will continue to accelerate, increasing the need for treatment, rehabilitation, and support.[8] Investments in research, diagnostics and companion diagnostics reflect this growing need in the biomarker space and an overall 13.3% expected CAGR in the next decade will need to be supported by a flexible and innovative platform.

Exosomes are data rich sources that are abundant, and given their nanoscale size (30 – 150 nm diameter), can pass through the blood-brain barrier[1,10] (Exosome release from cells shown in **Figure 2.0**). Exosomes are isolated from all biofluids, including serum, plasma, saliva, urine and cerebrospinal fluid; exosomes contain multiple components including lipids, proteins, message RNA (mRNA), long-non-coding RNA (lncRNA) and micro ribonucleic acids (miRNAs).[10] Tumor cells secrete more exosomes than normal cells and those exosomes secreted increase angiogenesis, promote tumor growth and can suppress immune cells to evade immune detection.[11-13] Other advantages include that exosomes are highly stable packages of RNA from the cell of origin and samples can be stored for up to 25 years in the freezer; allowing experimentation and analysis of RNA with tumor specific mutations in clinical trials spanning many years and opening the field to new opportunities. Exosomes make an attractive experimental approach as they limit time constraints between collection and analysis, are secreted increasingly from tumor cells, can pass the blood-brain barrier and are important communicators in tumor cell interactions.

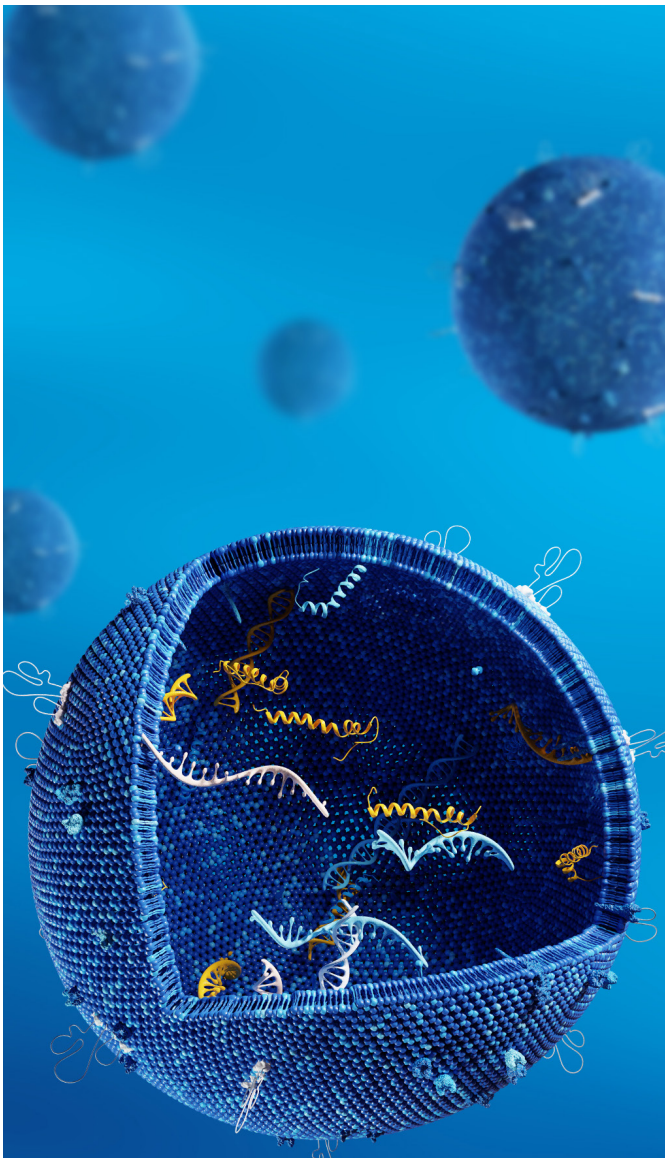


FIGURE 2 Exosomes being released from cells.

1. Accurately characterize and stratify patient populations

Exosomes provide a means of detecting evolving genomic changes relative to tumor progression using biofluid samples drawn over time. For example a critical element to some treatment therapies is Epidermal Growth Factor Receptor (EGFR). Detecting changes in EGFR can impact patient outcome as responders containing the EGFR target were 50 times more likely to respond to treatment compared to controls.[14] Full evidence of dynamic genomic characterization to stratify responders vs non-responders in Glioblastoma Multiforme (GBM) can be found in peer-reviewed work by Chi *et al.* in the Journal of Clinical Oncology, 2020.[5] Published GBM findings are supported by other research done in partnership with Exosome Diagnostics in **Figure 3.0** where genes were analyzed with the goal to stratify responders vs. non-responders. Patient stratification is one of the most critical elements of current therapeutic approaches and clinical trial design where selecting the appropriate patient can be the difference between success and failure.

Characterizing dynamic genomic changes is powerful but even more so when it is realized how broad and encompassing the genomic characterization can be. Independent studies on glioblastoma, demonstrated the multiple pathways that can impact responsiveness (**Figure 4.0**). In this sample cohort, 451 differentially expressed genes were found and in this specific case the focus was 25 of the most highly differentiated genes responsible for responsiveness to an inhibitor therapy. These genes as you can see in **Figure 4.0** applied to 8 major biological pathways and processes. Exosomes can reveal an abundance of information and analysis can characterize a variety of characteristics of interest.

2. Get versatility in target analytes to better characterize populations

Exosomes are data rich, containing information on numerous types of RNA, Protein and DNA. Data has been shown above for messenger RNA (mRNA) and long-non-coding RNA (lncRNA) in plasma/serum. In yet another independent partnership, we show the power of micro-RNAs

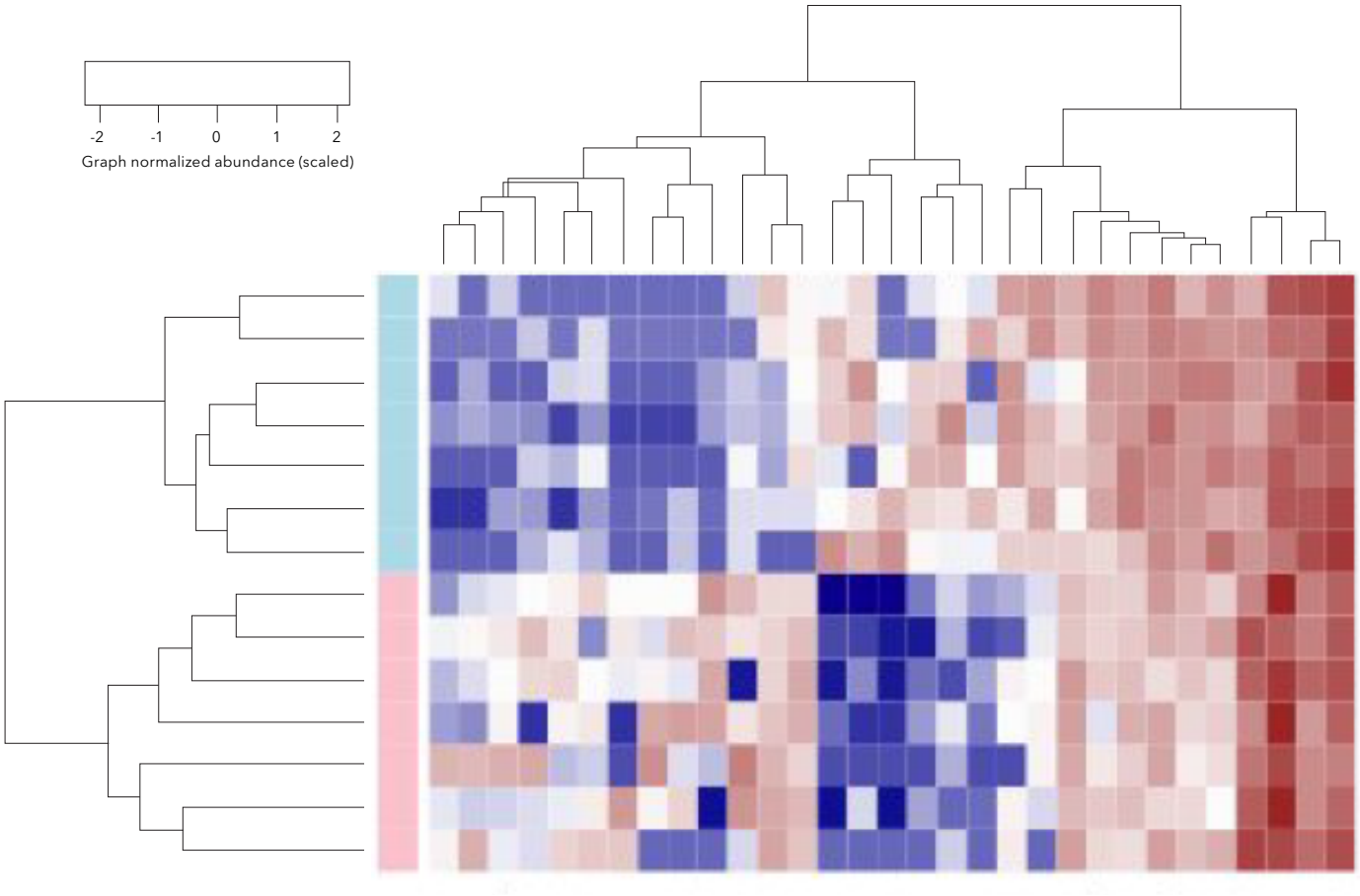


FIGURE 3 Responder vs non-responder. Glioblastoma patient samples and normal patient plasma are shown in each row. Samples were examined by long-RNA seq analysis (including mRNA and lncRNA) and found to have differentially expressed genes represented in each of the columns.

(mi-RNA) analysis in glioblastoma patients. MicroRNAs can affect the expression profiles of thousands of genes, their central role in disease has led to an explosion in the amount of peer-reviewed articles being published and the money put behind biomarker exploration.[15] **Figure 5.0** is a representative experiment on glioblastoma patient serum/plasma to stratify patient populations into responders and non-responders to a treatment. In this partnership/experiment, a single mi-RNA expression profile was capable of distinguishing responders vs. non-responders with relatively good accuracy. With data being shown for mRNA, lncRNA and miRNA it demonstrates the versatility of profiling with exosomal approaches.

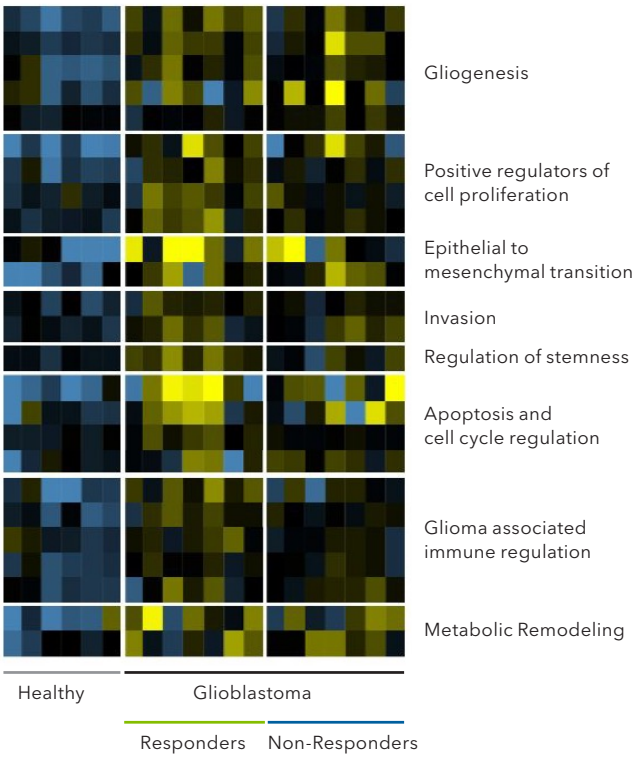
3. Analyze biofluids that can provide minimally to non-invasive options to obtain critical information

Plasma, urine, saliva and other biofluids including CSF can be used as the sample from which exosomes can be isolated and genomic profiling performed. As an example, in a glioblastoma study, CSF and plasma samples were obtained from the same patient and compared to further characterize what unique molecular profiles could be found. Analysis of serum and CSF samples are shown in **Figure 6.0**. GBM patients were compared, and 1,653 genes were found in CSF compared to plasma and 2,113 genes were found in plasma compared to CSF. The heat map in **Figure 6.0** demonstrates that CSF / plasma will have similarities and differences within the profiles as there are signals that overlap but there are unique signals for each biofluid.

FIGURE 4 Heat map depicting differential gene expression profiles of top 25 genes enriched in serum EV RNA of patients with GBM (n=14; responders, 7; non-responders, 7) as compared to healthy controls (n=6). Each column is a patient sample. Total reads were normalized to GAPDH expression. Top 285 enriched mRNAs were compared to the gene expression profiles of GBM and healthy tissue in The Cancer Genome Atlas (TCGA). Of the 65 mRNAs enriched in serum EVs and GBM tissue (TCGA), 25 genes were selected based on available relevant literature and cancer hallmarks.

4. Customized experimental approaches with complete quality control at each step

Customized experiments require complete quality control at each step. Reproducibility and repeatability in patient populations is a critical component to any partnership; exosomes can be isolated from CSF or plasma with repeatable and reproducible results (**Figure 7.0A/B**). The experiment shows total RNA replicates for two different patient samples, demonstrating variability across patient-to-patient samples but highly repeatable in technical replicates for the same sample. **Figure 7.0B** further illustrates consistency in one patient sample in the breakdown of RNA by biotype. Consistency of sample handling / processing or workflow does hold across several patient samples is shown in **Figure 7.0C** where spiked controls provide a validation of consistency from sample to sample. In a typical experimental design, to ensure repeated performance we perform spiked controls, replicates and at key stages verify qPCR within independent experiments; when required we include both a training and verification signature. Variability exists and the goal is to predict response based on characterizing those profiles but in order to do so a controlled environment must be the foundation.



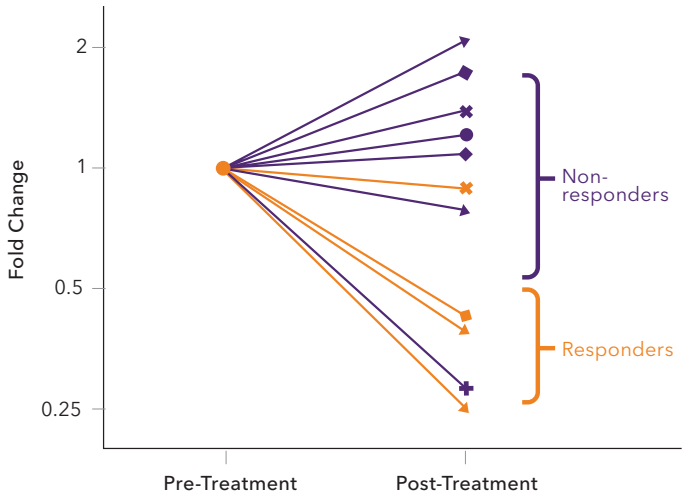


FIGURE 5 miRNA in glioblastoma. Analysis done by qPCR to analyze responders vs. non-responders based on the expression of one miRNA. Each line represents a patient and their pre-treatment and post-treatment expression levels.

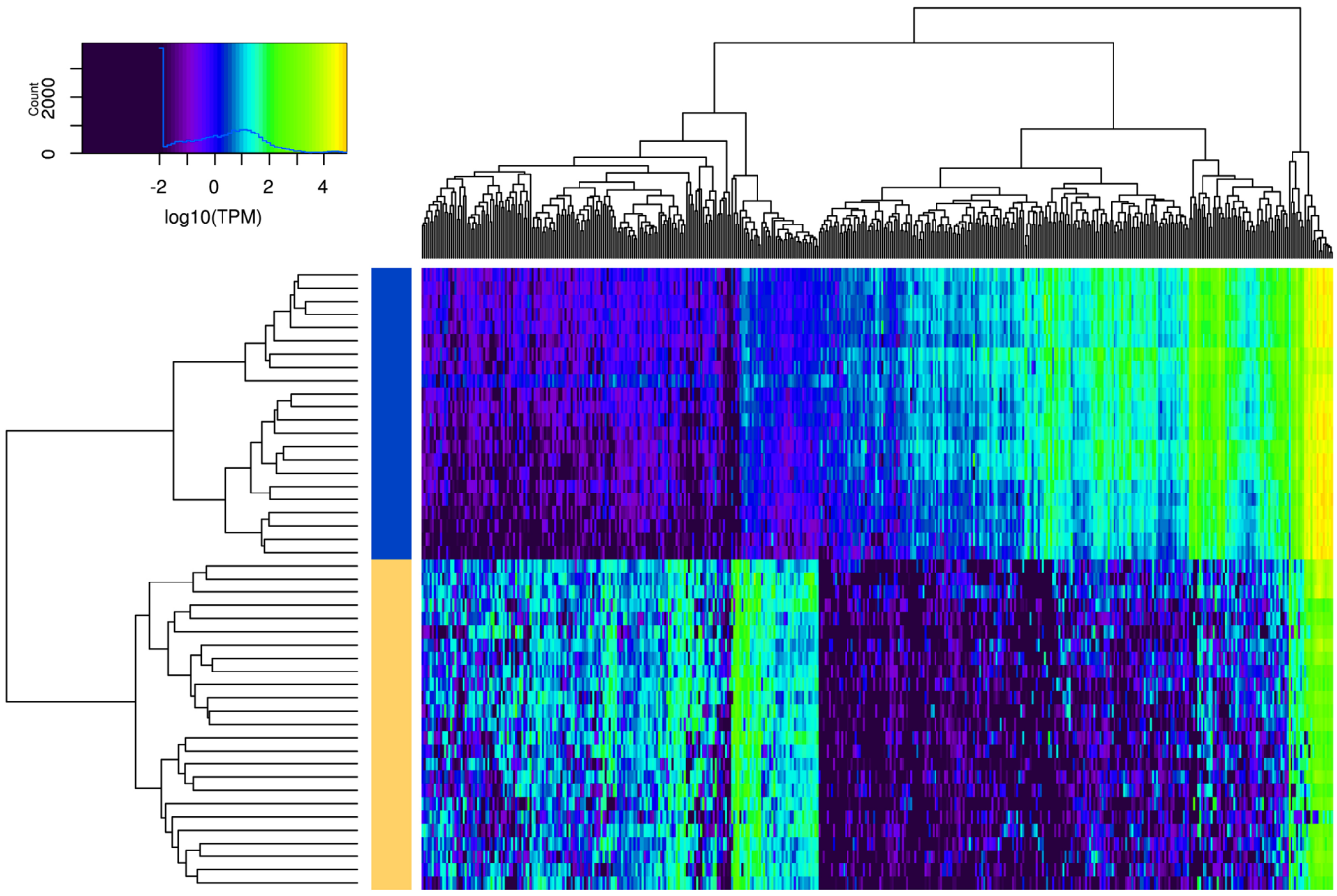


FIGURE 6 Differences in information from RNAs in Plasma vs CSF. The heat map is a breakdown in each row of thousands of mRNAs, Inc RNAs, pseudogenes, etc significantly differentially expressed between plasma and CSF. Many genes are specifically represented in only one of the biofluids. 1,653 were found in CSF>plasma and 2,113 in plasma > CSF.

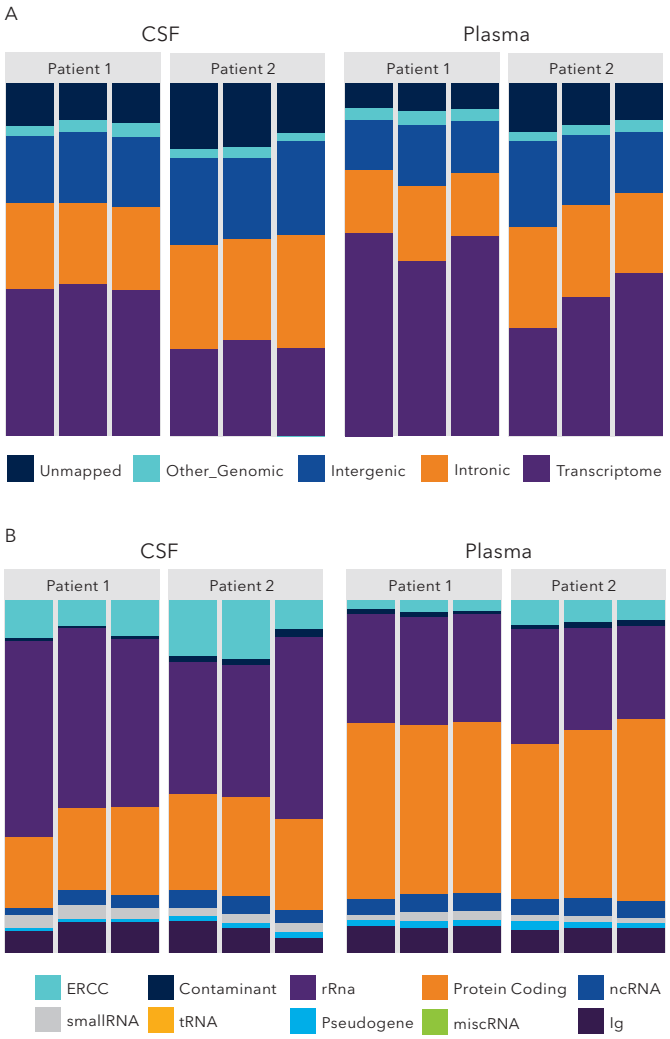


FIGURE 7 RNA-Seq quality control parameters that are critical to the quality of RNA biomarker discovery using the exosome platform. **A**) A high percentage of mappable sequences are analyzed in RNA-Seq that belong to the transcriptome aspect of genomic profiling; **B**) Across individuals and biofluids (plasma, CSF), there are significant and consistent proportions of protein-coding sequences, unique contents to biomarker discovery using exosomes for liquid biopsy; **C**) Excellent Pearson's correlation of ERCC spike-ins between samples demonstrates robust technical reproducibility of the RNA-Seq workflow.

Conclusions

Plasma, CSF and potentially other biofluids such as urine are all possible sample options that can be used to characterize various neurological disorders in more detail. Whether you are looking to predict patient responses to a therapy, understand resistance to a therapy or the potential for multiple segments of a patient population where they were once thought to be homogenous. The implications for exosomes in biomarker research are thought of as limitless and only bound currently by the research questions we pose and the funding to carry through. Neurological disorders call for increasingly customized experimental design and Exosome Diagnostics is uniquely suited with the patented technology, experience, and a pipeline already established to control for custom projects in a timely manner.

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